In the Specification

Page 1, after the title and before "Field of Invention", please insert the following paragraph.

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Application No. 09/586,216, filed June 2, 2000, which claims priority to Canadian Application No. 2,272,055, filed June 2, 1999, and to U.S. provisional Application No. 60/137,598, filed June 3, 1999.

Please amend the paragraph starting at page 2, line 19, as follows.

The invention includes a modified Gcc cDNA insert that can be inserted into any mammalian expression vector for use in the medical treatment of Gaucher disease. In a preferred embodiment, the modified cDNA was inserted into a vector named pINEX2.0 which was then used to transfect mammalian cells. When pINEX2.0 containing the unmodified Gcc cDNA coding sequence, pINEX5'GCC3', was transfected into cells, their RNA purified from cell lysates and subjected to reverse transcription followed by the polymerase chain reaction (RT-PCR), two distinct major bands were observed after agarose gel electrophoresis (Fig. 1). Isolation, purification and sequencing of the RT-PCR products identified a major aberrantly spliced mRNA species which encodes only a 19 amino acid peptide before encountering a STOP codon. Surprisingly, this aberrant splicing event occurred completely within the Gcc

cDNA coding sequence (Fig. 2), *i.e.* no vector sequences were involved. Site directed mutagenesis was performed to modify the nucleotide sequence in the region of aberrant mRNA splicing without affecting polypeptide coding (Fig. 3; SEQ ID NOS.: 2, 4, and 6). Modifications were aimed at disrupting the known consensus sequences for RNA-splicing (Krawczak et al. 1992). The effectiveness of these modifications were tested by transient transfection into CHO cells, followed by our human-specific immunoprecipitation assay for Gcc. Data (n=18) indicate a 5 ± 1(Std. Error)-fold increase in Gcc activity was achieved when the modified replaced the unmodified insert in the pINEX2.0 expression vector.

Please amend the paragraph starting at page 3, line 4, as follows.

The invention relates to an isolated Gcc DNA molecule, wherein the DNA molecule has a modification in at least one nucleotide that disrupts a splicing consensus sequence and prevents splicing of mRNA produced from the DNA molecule, while preserving the ability of the DNA to express active Gcc. The modification impairs a consensus nucleotide sequence needed to induce splicing. The DNA molecule is preferably modified at two cryptic splice sites. The DNA preferably includes a mutation in the 3' junction site. In one embodiment, the mutation is as shown in the 3' junction site (SEQ ID NO.: 18) in Table 1, or a functionally equivalent mutation. In another embodiment, the DNA molecule includes a mutation in the 5' splice junction

site. The mutation is preferably as shown in the 5' junction site (SEQ ID NO.: 19) in Table 1, or a functionally equivalent mutation.

Please amend the paragraph starting at page 3, line 13, as follows.

The DNA molecule preferably includes all or part of the nucleotide sequence shown in figure 4(b) (SEQ ID NO.: 13).

Please amend the paragraph starting at page 4, line 21, as follows.

Figure 3 (SEQ ID NOS.: 2, 4, and 6). Comparison of consensus splice site donor/acceptor site and "Cryptic" splice sites in Gcc cDNA. Sequences of (a) unmodified Gcc cDNA contained in pINEX5'Gcc3' (b) In a preferred embodiment, this sequence represents modified Gcc cDNA contained in pINEX-WEIRD. The translated amino acid sequence for either the modified or unmodified Gcc cDNAs is also given, note that the modified nucleotides had no effect on the amino acid sequence.

Please amend the paragraph starting at page 4, line 26, as follows.

Figure 4. (a) (SEQ ID NOS.: 11 and 12) The sequences of the aberrantly processed transcript from the unmodified Gcc cDNA insert in pINEX5'Gcc3' and its translated

polypeptide (b) (SEQ ID NOS.: 13 and 14) Modified DNA and its translated polypeptide. In a preferred embodiment, this sequence represents modified Gcc cDNA.

Please amend the paragraph starting at page 5, line 23, as follows.

During the search to improve the efficiency of human Gcc expression it was determined that a major amount of the RNA transcribed from any vector was aberrantly spliced due to cryptic 5' and 3' splice sites contained in the human Gcc cDNA (Fig. 1 & 2). Since this RNA species encodes only a 19 amino acid peptide, it is far less stable than the properly spliced product encoding the complete 536 residues of Gcc (Maquat 1996), and therefore transcribed at a much higher level than is indicated from our steady-state RT-PCR data (Fig. 1). We modified the two cryptic sites in a manner that conserved the wild type amino acid sequence while destroying the consensus nucleotide sequences needed to induce splicing (Fig. 3; SEQ ID NOS.: 2, 4, and 6). Transient expression of this modified insert indicated a 5-fold increase in Gcc expression. Such an increase in expression efficiency is not only valuable for any gene therapy approach, but also useful in decreasing the cost of enzyme replacement since the enzyme source is now Gcc-transfected mammalian cells.

Please amend the paragraph starting at page 6, line 9, as follows.

Other useful DNA inserts include a nucleic acid molecule having at least about: 50%, 60%, 70%, 80%, 90%, 95%, 99% or 99.5% sequence identity to the modified Gcc nucleic acid molecule (the Gcc sequence in figure 4(b) (SEQ ID NO.: 13)) wherein the molecule having sequence identity has a modification in at least one nucleotide (preferably two nucleotides) that disrupts a splicing consensus sequence and prevents splicing of mRNA while it encodes a polypeptide having Gcc activity. Changes in the Gcc nucleotide sequence which result in production of a chemically equivalent (for example, as a result of redundancy of the genetic code) or chemically similar amino acid (for example where sequence similarity is present), may also be made to produce high levels of unspliced transcript from the Gcc cDNA for therapeutic use. The DNA molecule or DNA molecule fragment may be isolated from a native source (in sense or antisense orientations) and modified or synthesized (with or without subsequent modification). It may be a mutated native or synthetic sequence or a combination of these in order to prevent or decrease aberrently spliced transcripts.

Please amend the paragraph starting at page 8, line 4, as follows.

Sequencing results confirmed that the major alternatively spliced species resulted from the removal of sequences within the Gcc cDNA itself, through the recognition of cryptic 5' and 3' splice sites roughly corresponding to the known consensus sequences that induce RNA splicing in mammalian cells (Krawczak et al. 1992). The deduced

amino acid sequence from this RNA species predicts a reading frame shift after Arg^{17} and an early stop two codons later (Fig. 3; SEQ ID NOS.: 2, 4, and 6). This would encode only a 19 amino acid peptide lacking even a complete signal sequence, necessary for targeting the protein to the cell's endoplasmic reticulum. In order to eliminate aberrant splicing, the Gcc cDNA was modified by site-directed mutagenesis to alter some of the critical nucleotides making up the consensus sequences (Krawczak et al. 1992) to ensure that the cryptic splice sites no longer be recognized by the RNA processing mechanism. Care was taken to preserve the amino acid coding sequence. Figure 3 (SEQ ID NOS.: 2, 4, and 6) shows the consensus sequence for the 5' or 3' splice junctions (Krawczak et al. 1992), the original nucleotide sequence of the Gcc cDNA, the deduced amino acid sequence, and the modifications undertaken to destroy the consensus splicing sequences. Other modifications to destroy the consensus splicing sequences will be apparent.

Please amend the paragraph starting at page 8, line 28, as follows.

Many modifications may be made to the vector and Gcc DNA sequences and these will be apparent to one skilled in the art. The invention includes nucleotide modifications of the sequences disclosed in this application (or fragments thereof) that are capable of expressing Gcc in *in vivo* or *in vitro* cells. For example, the regulatory sequences may be modified or a nucleic acid sequence to be expressed may be modified

using techniques known in the art. Modifications include substitution, insertion or deletion of nucleotides or altering the relative positions or order of nucleotides. The invention includes DNA which has a sequence with sufficient identity to a nucleotide sequence described in this application to hybridize under moderate to high stringency hybridization conditions. Hybridization techniques are well known in the art (see Sambrook et al. Molecular Cloning: A Laboratory Manual, Most Recent Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). High stringency washes have low salt (preferably about 0.2% SSC), and low stringency washes have high salt (preferably about 2% SSC). A temperature of about 37°C or about 42°C is considered low stringency, and a temperature of about 50-65°C is high stringency. The modified inserts encoding Gcc of the invention also include DNA molecules (or a fragment thereof) having at least 50% identity, at least 70% identity, at least 80% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or, most preferred, at least 99%, 99.5% or 99.8% identity to a modified Gcc nucleic acid molecule acid as shown in figure 4(a) (SEQ ID NO.: 11), which have a modified consensus sequence to prevent splicing and which are capable of expressing DNA molecules *in vivo* or *in vitro*. Identity refers to the similarity of two nucleotide sequences that are aligned so that the highest order match is obtained. Identity is calculated according to methods known in the art. For example, if a nucleotide sequence (called "Sequence A") has 90% identity to the Gcc sequence in figure 4(b) (SEQ ID NO.: 13)], then Sequence A will be identical to the referenced

portion of figure 4(b) (SEQ ID NO.: 13) except that Sequence A may include up to 10 point mutations (such as deletions or substitutions with other nucleotides) per each 100 nucleotides of the referenced portion of figure 4(b) (SEQ ID NO.: 13). The invention also includes DNA sequences which are complementary to the aforementioned sequences. "Sequence identity" may be determined, for example, by the Gap program. The algorithm of Needleman and Wunsch (1970 J Mol. Biol. 48:443-453) is used in the Gap program.

Please amend the paragraph starting at page 9, line 32, as follows.

Other functionally equivalent forms of the modified Gcc DNA of the invention can be identified using conventional DNA-DNA or DNA-RNA hybridization techniques. Thus, the present invention also includes nucleotide sequences that hybridize to the sequence in figure 4(b) (SEQ ID NO.: 13) or its complementary sequence, wherein the molecule that hybridizes to the Gcc portion in 4(b) (SEQ ID NO.: 13) has a modification in at least one nucleotide (more preferably at least two nucleotides) that disrupts a splicing consensus sequence and prevents aberrant splicing of mRNA while it encodes a polypeptide having Gcc activity. Such nucleic acid molecules preferably hybridize to the Gcc sequence in Figure 4(b) (SEQ ID NO.: 13) under moderate to high stringency conditions For example, high stringency washes have low salt (preferably about 0.2% SSC), and low stringency washes have high salt

(preferably about 2% SSC). A temperature of about 37°C or about 42°C is considered low stringency, and a temperature of about 50-65°C is high stringency (see Sambrook et al. Molecular Cloning: A Laboratory Manual, Most Recent Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

Please amend the paragraph starting at page 16, line 9, as follows.

Cells were grown in large dishes (P150), and RNA was isolated from control CHO cells and the A7 clone, according to the one-step guanidinium isothiocyanate procedure(Chomczynski and Sacchi 1987). RNA (1μg), primer (SPR2 (SEQ ID NO.: 15) (see Table 1), 200pmol), RNase inhibitor, and ddH_2O (to 12.5μL total), were mixed and incubated at 65°C for 20 minutes. After cooling on ice for 5 minutes, the remaining components of the RT reaction cocktail were added (RT buffer, DTT, dNTPs, RNase inhibitor, and reverse transcriptase). The reaction cocktail (total 25μL) was incubated at 37°C for 90 min.

Please amend the paragraph starting at page 16, line 16, as follows.

PCR was performed using the RT reaction products (1μL) as template. After addition of ddH_20, and primers (SPF (SEQ ID NO.: 16) and 53GCC2000R (SEQ ID

NO.: 17) (see Table 1), 20pmol each), the reaction was incubated at 95°C for 5 minutes to inactivate the reverse transcriptase. The remaining reaction components (dNTPs, MgCl2, and Taq polymerase (Gibco BRL)) were used at manufacturers suggested levels. Thermocycling was performed under the following conditions: 94°C/3min; 30 cycles of 94°/1.5min, 55°/1min, 72°/1.5min; 72°/10min. Samples of the PCR reaction (10μL) were loaded onto a 1.5% agarose gel using Tris-Acetate-EDTA buffer (TAE, 40mM Tris-acetate / 2mM EDTA), electrophoresed and visualized using ethidium bromide.

Please amend the paragraph starting at page 17, line 4, as follows.

The cryptic splice site located within the Gcc cDNA was modified by site-directed mutagenesis in order to remove potential consensus splice junction sites from the Gcc cDNA. A PCR product was obtained using one oligonucleotide primer which mutagenized a number of bases in the putative 3' junction site (SEQ ID NO.: 18) (3'-junction (see Table 1)) and another for the putative 5'-splice junction site (SEQ ID NO.: 19) (5'-junction (see Table 1)). The PCR reaction contained: 1X Pfu reaction buffer (10X stock provided by manufacturer), 0.4mM dNTP, 10ng template DNA (pINEX-5'-GCC-3'), 500ng of each oligo, and 2.5U Pfu DNA Polymerase in a final volume of 50μL in the appropriate buffer.

Please amend the paragraph starting at page 17, line 12, as follows.

Amplification was performed using a Robocycler 40 Temperature Cycler (Stratagene) for 30 cycles, with temperatures and times as follows: 94°C/45 sec., 59°C / 1 min. and 72°C / 1 min. 20 sec. The PCR product was used as a mega-primer in the second round of PCR. The second PCR reaction consisted of: 5μL of the above PCR reaction mixture, 1X Pfu reaction buffer (10X stock provided by manufacturer), 0.4mM dNTPs, 50 ng template (pINEX-5'-GCC-3'), 500ng upstream oligo (SPF; SEQ ID NO.: 16) and 5U Pfu DNA polymerase in a final reaction volume of 100μL. Reaction temperature conditions used were the same as for the initial PCR above. The PCR products were digested with 10U of Dra III and Xho I for 3 hr at 37°C. The plasmid pINEX-5'-GCC-3' was digested in parallel using the same method. Digested products were electrophoretically separated on an agarose gel, and the appropriate pieces were excised and purified as described above. Ligation was performed in a 20μL final volume using 5U of T4 DNA Ligase (MBI Fermentas, Lithuania), incubating overnight at 16°C to produce pINEX-WEIRD. DNA was transformed into DH5a E. coli cells (Gibco BRL) and plated onto appropriate LB agar plates containing antibiotics. Plasmid DNA was isolated and screened by restriction digest and sequencing to confirm that they contained the appropriate insert.

Please amend Table 1 on page 21 as follows.

TABLE 1: Sequence of Oligos Used in this Study:

Oligo Name	Oligo Sequence* (5' to 3')
SPR2 (SEQ ID NO.: 15)	GCCAGTGTGATGGATATCTGC
SPF (SEQ ID NO.: 16)	GACCGATCCAGCCTCCGGACTCT
53GCC2000R (SEQ ID NO.: 17)	GCCGCACACTCTGCTCCCAGAA
3-junction (SEQ ID NO.: 18)	CATCCGTCGCCCACTGCGTGTACTCTCATAGCGGGAAAATG TTCCCCAGGGCAGG
5'junction (SEQ ID NO.: 19)	CCTTTGAGTAGAGTCTCCATCATGGCTGGC

= Bases underlined indicate bases changed in site-directed mutagenesis PCR procedures.